Aquaculture Research, 2006, **37**, 299–301 doi: 10.1111/j.1365-2109.2005.01416.x

SHORT COMMUNICATION

Detection of off-flavour in channel catfish (*Ictalurus punctatus* Rafinesque) fillets by trained dogs

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'Off-flavour' is problematic for producers of channel catfish *Ictalurus punctatus* (Raffinesque) in the southeastern United States, which is caused by the uptake and accumulation in the catfish flesh of the metabolites geosmin (GSM) and 2-methylisoborneol (MIB) respectively. These compounds are produced by certain species of cyanobacteria, or blue–green algae, that grow in the catfish aquaculture ponds and are most abundant during the summer and early autumn months. The catfish industry may lose as much as 23 million $US annually (Hanson 2003) to off-flavour problems. Although GSM and MIB are not harmful to the consumer, the unpleasant tastes produced by these compounds must be detected by processors to help prevent unpalatable catfish from reaching the marketplace and leading to consumer dissatisfaction and future avoidance of their product. Normally, processors employ trained human sensory personnel who will taste-test a fillet sample from each pond to be harvested. The processor will only accept the catfish after they are deemed to be 'on-flavour', or without detectable unpleasant taste or odour. Both GSM and MIB can also be detected by using analytical/chemical methods. Solid phase microextraction-gas chromatography with mass spectrometry detection (SPME/GC/MS) can be used to identify and quantify these compounds in water (Lloyd, Lea, Zimba & Grimm 1998; Watson, Brownlee, Satchwill & Hargesheimer 2000) and fish tissue (Grimm, Lloyd, Batista & Zimba 2000). These methods are too expensive and time consuming to be used on a regular basis by processors. In practice, human sensory personnel are relied upon to detect and determine the intensity of earthy-musty off-flavours. The human detection threshold for these compounds in catfish flesh has been placed variously at 700 ng kg^{-1} by the average consumer (Persson 1980; Johnsen & Kelly 1990) to as low as 100 ng kg^{-1} for a trained flavour tester (Grimm, Lloyd & Zimba 2004).

We demonstrated in a previous study that trained dogs could be used to detect GSM and MIB in pond water (Shelby, Schrader, Tucker, Klesius & Myers 2004). The purpose of the current study was to determine if the dogs which had been trained in the earlier study to detect GSM and MIB in purified water and pond water could also be used to detect these off-flavour compounds in the processed product.

The protocol used for the tests was identical to that described previously (Shelby *et al.* 2004). Two male and two female mixed-breed adult dogs which were obtained from the local animal shelter and trained to detect GSM and MIB in water samples were used in these tests (Table 1). Samples of catfish were obtained from commercial ponds in west-central Mississippi in 2002, and fillets of these catfish were analysed by SPME-GC/-MS (Grimm *et al.* 2000). Several of the samples were found to be redundant with regard to levels of MIB and GSM, and were not presented to the dogs.

Table 1 Characteristics of dogs used in the off-flavour detection study

Dog	Sex	Breed	Approximate age (years)	Years in program
Rusty	M	Labrador Retriever mix	4	2
Maggie	F	German Shepherd mix	4	2
Ralph	M	Setter mix	2	1
Ginger	F	Chow mix	3	2

F, female; M, male.

Dogs were initially trained using analytical standards of GSM and MIB in purified water (Shelby *et al.* 2004), and eventually pond water containing naturally occurring levels of both compounds. Training and testing protocol was identical to that previously used for water samples. Fillet samples were frozen ($-80\,^{\circ}C$) after SPME-GC-MS analysis and remained so until presented to the dogs. Fillet tissue was removed from the same anatomical site of each fillet (e.g., abdomen region), cut into 1 cm^3 pieces to fit into 1.5 mL microcentrifuge tubes, and then allowed to thaw for 15 min prior to testing by the dogs. One tube was placed in each 10×10 cm steel electrical outlet box. Five boxes were placed in openings in a plywood wall spaced 46 cm apart and 46 cm above the floor. One off-flavour sample box was randomly placed on the wall with four on-flavour fillet sample boxes. The same nontarget on-flavour sample was used in the four boxes for all tests. Boxes were not fixed, and were randomly repositioned for each pass. Dogs were led past the boxes, trained to smell each box and to sit if the target scent was encountered. Correct responses were rewarded with a small food treat and praise. Incorrect responses were admonished with a 'no' from the handler. Daily sessions consisted of 20 sequential passes for each dog, for which a percentage of correct responses was tabulated. This required approximately 30 min for each dog. Each session began with three passes in which the dog was shown the target sample and rewarded for sitting. After this initial instruction, the human handler was blind to the target box location, while an assistant randomized the boxes. This protocol was used to avoid visual clues from the handler being detected by the dog. We began with the fillet samples having the highest concentrations of MIB and GSM and then proceeded to test each dog with each sample in this manner until the lowest concentrations were tested. Levels of GSM and MIB in the fillets were considered typical for catfish obtained from west Mississippi commercial catfish ponds, and these catfish samples included GSM and MIB together in varying concentrations, MIB only and GSM only. In the final series of tests, we included, as the target sample, a fillet which had been determined to be 'negative' for GSM and MIB by SPME-GC-MS.

As the target sample was placed randomly among four different on-flavour samples, each sample had an equal probability because of chance of being selected by the dog. The χ^2 statistic was used to calculate deviation from the expected value of 1/5 (20%) for any sample being selected.

The mean combined correct response rate for all dogs and all samples was 81%. The percentage of correct responses did not appear to decrease with decreasing levels of GSM or MIB. In fact, Ralph and Ginger actually improved in their ability to detect the target off-flavour sample as levels of GSM and MIB declined. Geosmin and MIB were detected with equal accuracy regardless of which compound or the level of the compound that was present. The exception was when one on-flavour sample was placed with four different on-flavour nontarget samples. In this case, most dogs had difficulty in detecting the 'different' sample. However, even for this sample, the total correct responses was significantly ($P \geq 0.05$) greater than would be expected because of chance alone (20%). Ginger, in fact, detected this sample with 90% accuracy.

The most surprising result of our study is that involving sample 1 (Table 2) in which both the target and nontarget fillet samples came from ponds which were determined to be on-flavour by SPME-GC-MS. All of the dogs were able to identify the single 'different' sample with a mean accuracy of 53%. We attribute these results to the ability of the dogs to distinguish different on-flavour samples because of subtle variations in other odours. As these catfish fillet samples were obtained from different ponds at different times with different water sources, and possibly different genetic stock, one might expect variation in the chemical composition of the muscle tissue. Another possibility is that one or both of these compounds was present but at a level below 1 ng L^{-1}, the detection threshold level of the analytical equipment used in this study.

We do not propose that dogs replace humans as 'taste-testers' at catfish processing facilities for several reasons. A false-negative test could result if a unique unpleasant flavour, for which the dogs had not been trained, were encountered in a sample. Human sensory personnel would recognize and interdict

Table 2 Cumulative percentage of correct responses in distinguishing between on-flavour (non-target) and off-flavour (target) catfish fillet samples for four dogs over a 7-week testing period

	GC/MS*		Rusty		Maggie		Ralph		Ginger	
Sample	MIB	GSM	%	Total	%	Total	%	Total	%	Total
13	3534	1206	73	40	88	40	70	20	85	20
12	1096	0	88	40	100	40	90	20	75	20
11	1095	0	53	40	90	40	70	20	100	20
10	22	439	65	20	75	20	95	20	83	40
9	13	321	70	20	75	20	90	20	60	20
7	262	0	50	20	80	20	100	20	100	20
6	120	0	95	20	85	20	100	20	95	20
5	0	114	90	20	85	20	100	20	95	20
1	0	0	50	20	38	20	32	60	90	40

*Methylisoborneol (MIB) and geosmin (GSM) concentrations (ng L^{-1}) were determined by gas chromatography-mass spectrometry (GC-MS). Percentages were calculated by total correct responses divided by total responses with a one out of five (20% random) choice of one off-flavour fillet sample randomized with four on-flavour fillet samples. Total numbers of tests are also indicated. Target sample '1' was determined to be on-flavour, but was tested using the non-target on-flavour sample used in all other tests.

these fish. Similarly, a unique off-flavour might seem agreeable, or even pleasant to the dog and not be identified as off-flavour. The detection of GSM and MIB in catfish fillets can be useful for processors and producers in those cases where the off-flavours compounds no longer persist in the pond water but are still detectable in the catfish flesh because of the incomplete depuration of GSM and MIB to levels that are deemed acceptable for market. A more practical application of this technology would be to use trained dogs to test actual pond water samples. This approach would enable the producer to be aware of the development of off-flavour problems earlier, and take management actions (e.g., application of algicides) to help reduce and eventually eliminate the accumulation of off-flavour compounds in the farm-raised catfish.

Acknowledgments

We thank Erin Arthur, Erica Blackman, Jason Bonner, Julie Fetzer-Gill, Lindsey Mayo, Anna Moore, Ramona Pace, Phaedra Page, Ning Qin, Rebecca Robinson and Katherine Ragland, Kelly Shanley, Greg Whitis and April Womack for technical and other assistance.

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Keywords: catfish, off-flavour, 2-methylisoborneol, geosmin, canine olfaction

